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# Effect of cattle urine addition on the surface emissions and subsurface concentrations of greenhouse gases in a UK peat grassland



A. Boon<sup>a,b,\*</sup>, J.S. Robinson<sup>a</sup>, D.R. Chadwick<sup>b,c</sup>, L.M. Cardenas<sup>b</sup>

<sup>a</sup> Department of Geography and Environmental Science, University of Reading, Whiteknights, P.O. Box 233, Reading, RG6 6DW, United Kingdom

<sup>b</sup> Rothamsted Research North Wyke, Okehampton, Devon, EX20 2SB, United Kingdom

<sup>c</sup> Environment Centre Wales, Deiniol Rd., Bangor University, Bangor LL57 2UW, United Kingdom

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## ABSTRACT

Grazing systems represent a substantial percentage of the global anthropogenic flux of nitrous oxide ( $\text{N}_2\text{O}$ ) as a result of nitrogen addition to the soil. The pool of available carbon that is added to the soil from livestock excreta also provides substrate for the production of carbon dioxide ( $\text{CO}_2$ ) and methane ( $\text{CH}_4$ ) by soil microorganisms. A study into the production and emission of  $\text{CO}_2$ ,  $\text{CH}_4$  and  $\text{N}_2\text{O}$  from cattle urine amended pasture was carried out on the Somerset Levels and Moors, UK over a three-month period. Urine-amended plots ( $50 \text{ g N m}^{-2}$ ) were compared to control plots to which only water ( $12 \text{ mg N m}^{-2}$ ) was applied.  $\text{CO}_2$  emission peaked at  $5200 \text{ mg CO}_2 \text{ m}^{-2} \text{ d}^{-1}$  directly after application.  $\text{CH}_4$  flux decreased to  $-2000 \mu\text{g CH}_4 \text{ m}^{-2} \text{ d}^{-1}$  two days after application; however, net  $\text{CH}_4$  flux was positive from urine treated plots and negative from control plots.  $\text{N}_2\text{O}$  emission peaked at  $88 \text{ mg N}_2\text{O m}^{-2} \text{ d}^{-1}$  12 days after application. Subsurface  $\text{CH}_4$  and  $\text{N}_2\text{O}$  concentrations were higher in the urine treated plots than the controls. There was no effect of treatment on subsurface  $\text{CO}_2$  concentrations. Subsurface  $\text{N}_2\text{O}$  peaked at 500 ppm 12 days after and 1200 ppm 56 days after application. Subsurface  $\text{NO}_3^-$  concentration peaked at approximately  $300 \text{ mg N kg dry soil}^{-1}$  12 days after application. Results indicate that denitrification is the key driver for  $\text{N}_2\text{O}$  release in peatlands and that this production is strongly related to rainfall events and water-table movement.  $\text{N}_2\text{O}$  production at depth continued long after emissions were detected at the surface. Further understanding of the interaction between subsurface gas concentrations, surface emissions and soil hydrological conditions is required to successfully predict greenhouse gas production and emission.

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## 1. Introduction

Nitrous oxide ( $\text{N}_2\text{O}$ ) is an important greenhouse gas (GHG) with 298 times the Global Warming Potential (GWP) of  $\text{CO}_2$  (Forster et al., 2007).  $\text{N}_2\text{O}$  is produced as a result of microbial processes operating in the soil profile, whereby it is a by-product of the reduction of nitrate ( $\text{NO}_3^-$ ) to nitrogen gas ( $\text{N}_2$ ) (denitrification), the ammonification of nitrate and the oxidation of ammonium ( $\text{NH}_4^+$ ) to  $\text{NO}_3^-$  (nitrification) (Firestone et al., 1980; Baggs, 2011). Agricultural systems, comprising both livestock and arable production, return substantial amounts of mineral

N to the soil and therefore contribute significantly to global emissions of  $\text{N}_2\text{O}$  (IPCC, 2001). Grazing systems are thought to represent 16% of the global anthropogenic flux of  $\text{N}_2\text{O}$  (IPCC, 2001) as livestock add nitrogen (N) to the soil in the form of excreta.

Cattle urine has been shown to stimulate  $\text{N}_2\text{O}$  production to a larger extent than dung due to the dual effect of a large pool of readily available N and C and increased soil water content (e.g. Allen et al., 1996; van Groenigen et al., 2005a). Cattle urine supplies greater amounts of N to the patch than the pasture N demand, thereby facilitating losses through leaching and gaseous emissions (Di and Cameron, 2002). Cattle urine N content varies between 1 and  $20 \text{ g L}^{-1}$  due to differences in water intake and diet (Oenema et al., 1997; Leterme et al., 2003) and is on average  $6 \text{ g N L}^{-1}$  (Leterme et al., 2003; Bristow et al., 1992). Urine patch radius is generally around 0.32–0.35 m but ranges between 0.1 and 0.6 m for dairy cattle (Moir et al., 2011). The surface area of urine patches is generally between 0.34 and  $0.40 \text{ m}^2$  (Moir et al., 2011; Oenema et al., 1997) giving rise to an N deposition of 20–80  $\text{g N m}^{-2}$  (200–800  $\text{kg N ha}^{-1}$ ) on each urination event (Oenema et al., 1997;

\* Corresponding author at: Department of Meteorology, University of Reading, Earley Gate, Reading, RG6 6BB, United Kingdom. Tel.: +44 0 118 378 7060.

E-mail addresses: [alex.boon@reading.ac.uk](mailto:alex.boon@reading.ac.uk) (A. Boon), [j.s.robinson@reading.ac.uk](mailto:j.s.robinson@reading.ac.uk) (J.S. Robinson), [d.chadwick@bangor.ac.uk](mailto:d.chadwick@bangor.ac.uk) (D.R. Chadwick), [laura.cardenas@rothamsted.ac.uk](mailto:laura.cardenas@rothamsted.ac.uk) (L.M. Cardenas).

Whitehead, 1986). For beef cattle urine the typical N loading is 700 kg N ha<sup>-1</sup> (Haynes and Williams, 1993).

On contact with the soil, urea-N is rapidly hydrolysed to ammonia (NH<sub>3</sub>), catalysed by the enzyme urease which is ubiquitous in soils as a result of microbial activity. This process is dependent. The hydrolysis process also reduces the available carbon from the urea, as CO<sub>2</sub> is a by-product of the reaction. Hydrolysis can account for over 50% of the added urine-C depending on soil moisture (Lambie et al., 2013). The remaining C provides a substrate for respiration (and therefore emission of CO<sub>2</sub>) or for CH<sub>4</sub> production in anoxic soils (Yamulki et al., 1999; Liebig et al., 2008). Studies have also shown that addition of cattle urine can increase the solubility of soil C, leading to increased soil C decomposition and therefore potentially increased CO<sub>2</sub> emission (Clough et al., 2003a) and leaching (Lambie et al., 2012). In addition to potential for increased N<sub>2</sub>O and CO<sub>2</sub> production in urine patched, NH<sub>4</sub><sup>+</sup> is known to inhibit oxidation of CH<sub>4</sub> and therefore promote increased CH<sub>4</sub> emission (Mosier et al., 1991; Dobbie and Smith, 1996).

Studies indicate that even short-term grazing can cause a significant increase in N<sub>2</sub>O emissions, particularly when combined with compaction and seasonal water-table rise (van Groenigen et al., 2005b; van Beek et al., 2011). There is a wide body of research into the effect of cattle excreta on soils, with focuses on soil moisture, N content, urine volume and interactions with dung and fertilisers (e.g. Allen et al., 1996; Velthof et al., 1996; van Groenigen et al., 2005a,b; Maljanen et al., 2007) but few focus exclusively on peat soils (Koops et al., 1997; van Beek et al., 2011) and few include observations of all three greenhouse gases under urine patches (Liebig et al., 2008; Lin et al., 2009). Peat soils by definition have higher organic matter content than mineral soils. This leads to physical differences between peat and mineral soils; in particular higher porosity and gas diffusion coefficient (Boon et al., 2013). Additionally, due to the tendency of peat soils shrink and swell with changing soil moisture, they exhibit strong variations soil hydraulic properties such as moisture retention (Kechavarzi et al., 2010) compared to mineral soils. Peat soils also generally have higher mineralisation rates than mineral soils leading to higher available N, which combined with higher moisture retention leads to increased N<sub>2</sub>O emission through denitrification (Koops et al., 1997). Peat soils have been shown to have increased N<sub>2</sub>O emissions with respect to mineral soils as a result of a combination of these factors, particularly when amended with fertilisers or livestock excreta (Velthof and Oenema, 1995). Due to the increased availability of soil organic carbon, peat soils are substantial sources of CH<sub>4</sub> when in an anaerobic state and CO<sub>2</sub> when in an aerobic state (Moore and Dalva, 1993).

Subsurface concentrations of greenhouse gases, when combined with measurements of soil nitrogen and carbon, can be used to identify the key processes contributing to the accumulation of gases that may be subsequently emitted to the surface (Li and Kelliher, 2005; Li and Kelliher, 2007). These measurements can be used to determine zones of production and storage of greenhouse gases in the soil, particularly when combined with soil physical measurements such as bulk density, air-filled porosity and the gas diffusion coefficient, all important predictors of greenhouse gas emissions (Ball, 2013; Balaine et al., 2013). Measurements of subsurface greenhouse gases are currently limited from peat soils (e.g. Clark et al., 2001; Elberling et al., 2011), particularly when these soils are subjected to agricultural amendments, and especially where measurements have been made of soil physical parameters.

Many lowland peatland environments in the UK are under seasonal grazing management, often as a contribution to conservation management schemes on tenanted farmland or nature reserves. Sheep production is regularly practiced on 85% of UK upland peat; but cattle and ponies are being introduced to manage fen vegetation

**Table 1**  
Characterisation of field soil (dry weight basis) between 0 and 30 cm depth (averaged data ± SE) (data collected May 2009–June 2010).

	Soil depth		
	0–10 cm	10–20 cm	20–30 cm
Texture	Clay loam	Loamy clay	Peat
Total C (%)	23.84 ± 0.74	16.08 ± 0.75	26.35 ± 2.08
Total N (%)	2.05 ± 0.05	1.54 ± 0.04	2.02 ± 0.12
C/N ratio	11.63 ± 0.15	10.41 ± 0.21	12.84 ± 0.48
SOM content (%)	48.57 ± 2.09	63.07 ± 1.45	43.13 ± 3.98
pH	5.05 ± 0.14	5.46 ± 0.15	5.57 ± 0.15
Bulk density (g soil cm <sup>-3</sup> )	0.44 ± 0.01	0.40 ± 0.01	0.14 ± 0.002

in lowland peatland and little study of the potential effect on GHG budgets for these environments has been conducted (Worrall et al., 2011). In this study, we aim to simulate small urination events on an area of UK peat grassland that is intensively grazed by beef steers for short period of time during autumn seasonal water-table rise. The main objective of this experiment was to quantify the difference between subsurface concentrations and surface fluxes of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O in plots treated with cattle urine and control plots treated with water. Secondary objectives were to examine the relative importance of water-table depth (WTD), water soluble (available) carbon (WSOC) and soil NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concentrations on CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O production and emission and thereby draw conclusions on the dominant greenhouse gas producing processes during short term cattle grazing on peat soils. We also consider the importance of measured soil physical parameters (porosity, bulk density and gas diffusion coefficient) for transport of greenhouse gases from the surface layers of soil to the atmosphere. We hypothesise that addition of cattle urine to the soil will produce significant differences in GHGs relative to the control plots and that water-table depth is the key control on these processes throughout the autumn rewetting period.

## 2. Materials and methods

### 2.1. Site description

The experimental site was located at West Sedgemoor, Somerset in SW England, UK (51°0.11'N, 2°55.16'W); a 1035 ha peatland site that forms part of the Somerset Levels and Moors Environmentally Sensitive Area (ESA). The site is managed by the Royal Society for the Protection of Birds (RSPB) for wetland bird conservation with the majority of land grazed in rotation with hay cutting a minimum of one year in three. The land is grazed by mixed breed beef steers belonging to a single tenanted farm holding. Approximately 30 animals graze 4.2 ha of land in rotation for two weeks in early autumn. It is known that little or no organic or inorganic fertiliser has been applied to the site for over 20 years.

The climate of the region is characterised by warm winters and cool summers with an average rainfall of 1005 mm annually and an average annual temperature of 10 °C. According to Findlay et al. (1984) and Heathwaite and Ross (1987), the soil profile of West Sedgemoor comprises three clear horizons within the surface 0–30 cm. The uppermost horizon between 0 and approximately 10 cm is loamy clay, resulting from the decay of surface vegetation and is significant despite cutting/grazing activity. Beneath this is a deposit of silty clay arising from periodic inundation by the nearby River Parrett. Below the clay, at between 25 and 30 cm depth in most cases, is black fibrous sedge peat (fibric histosol) of up to 8 m depth. Characterisation of the soil is given in Table 1. These data were collected as part of a separate field trial conducted between May 2009 and June 2010.

The selected field has its water-table controlled by two different drainage ditch management practices. The north and west ditches

are managed by the Parrett Internal Drainage Board (IDB) and the south and east ditches are managed by the RSPB for wetland conservation. The water-level is maintained in the IDB ditches via a large inlet flow into the River Parrett, which lies approximately 2.5 km to the east of the study field. The RSPB ditches are separated from the IDB ditches via a blockade at the north end of the south ditch and a removable pipe at the end of the east ditch that connects the two systems during times of high water level in both ditches but isolates them when the IDB ditches are drained. The IDB-managed ditches have a lower water level than the RSPB ditches between December and March for flood prevention and drainage of agricultural land and are higher between April and June. During the period of this research it was considered unlikely that there would be significant difference in water-table across the field. Kechavarzi et al. (2007) showed that without installation of subsurface irrigation channels, the ditch water level did not have a significant impact on the water-table towards the centre of the field.

The plant community on the experimental site is classified as MG8 according to the National Vegetation Classification (Rodwell, 1992). The MG8 vegetation community is described as a species-rich, varied water meadow with no particular dominant species but grasses accounting for most of the cover.

## 2.2. Experimental approach

Cattle urine was supplied by the Centre for Dairy Research (CEDAR) dairy unit (University of Reading) and stored unacidified for two weeks at  $-5^{\circ}\text{C}$  before application. The urine was thawed over a period of 48 h prior to application. The control application was water collected from one of the ditches surrounding the field. Prior to application, urine and ditch water samples were analysed for Total N and Total C using a Skalar 5000-02 Autoanalyser for N analyses and a Skalar Formacs<sup>HT</sup> TOC Analyser for C analyses. Urine total N was  $6.7 \pm 1.5 \text{ g L}^{-1}$  and total C was  $13.9 \pm 0.5 \text{ g L}^{-1}$ . Total N and C in the ditchwater were negligible in comparison ( $2.5 \pm 0.0$  and  $66.0 \pm 0.1 \text{ mg L}^{-1}$  respectively).

Two rows of five  $2 \text{ m}^2$  replicated plots, each 5 m apart with 2 m between each row, were set up in the field on 15/09/2010. These were placed approximately 5 m from the east ditch, which is managed for high water-table during the summer by preventing drainage into a wider channel leading to the river. An area of relatively low water-table fluctuation was chosen based upon results from a previous field study in order to improve the replication of the treatment and control plots. Each plot comprised a static chamber (described below) and three soil atmosphere samplers inserted at 10, 20 and 30 cm depth in the profile. The static chamber and soil atmosphere samplers were offset by approximately 0.75 m within each plot to ensure soil disturbance did not affect the chamber measurements. Treatment and control plots were placed 2 m apart and each adjacent pair of plots shared a dipwell, placed one metre from each plot.

One week following installation of the chambers and soil atmosphere samplers, treatments were applied to the plots (22/09/10). The  $5 \text{ L m}^{-2}$  treatments were applied in marked quadrants of the  $2 \text{ m}^2$  area using a watering can with a sprinkler. The full area of the plots was covered with urine in order to ensure the comparability of soil under the chamber, soil surrounding the subsurface samplers and the area of soil that was taken for analysis throughout the experimental period. The urine application rate was approximately  $49.8 \text{ g N m}^{-2}$  and  $65.2 \text{ g organic C m}^{-2}$  (equivalent to an N loading of  $498 \text{ kg ha}^{-1}$  and a C loading of  $652 \text{ kg ha}^{-1}$ ), appropriate to the average N contents reported in Oenema et al. (1997) and Leterme et al. (2003). The loading is lower than the expected figure given by Haynes and Williams (1993); however, it is within the range of typical values expressed by Oenema et al. (1997) and Whitehead (1986).

Analysis of the N and C content of the ditch water used on the control plots gave an application rate of  $12.4 \text{ mg N m}^{-2}$  and  $105 \text{ mg C m}^{-2}$  (equivalent to an N loading of  $0.124 \text{ kg ha}^{-1}$  and a C loading of  $1.05 \text{ kg ha}^{-1}$ ).

During the first two weeks following application, the plots were monitored for water-table depth and sampled for  $\text{CO}_2$ ,  $\text{CH}_4$  and  $\text{N}_2\text{O}$  emissions (chambers) and subsurface concentrations (soil atmosphere samplers) on six occasions (three times per week). Subsequently, monitoring and sampling took place every two weeks for two months. Samples were taken from static chambers to determine the surface fluxes of  $\text{N}_2\text{O}$ ,  $\text{CO}_2$  and  $\text{CH}_4$ , and from soil atmosphere samplers to determine the below ground concentrations of the gases. Soil was periodically sampled for WSOC,  $\text{NH}_4^+$  and Total Oxidised Nitrogen (TON). Meteorological data (maximum and minimum daily air temperature and rainfall) were collected three to four times a week from a meteorological station located on West Sedgemoor, within 2 km of the field site.

## 2.3. Gas sampling and analysis

The static chamber method (Mosier, 1989; Hutchinson and Livingston, 2002) was used to measure fluxes. Chambers were  $0.4 \text{ m} \times 0.4 \text{ m} \times 0.25 \text{ m}$  (internal dimensions) white plastic boxes with gas tight lids (Cardenas et al., 2010). A specialised cutting tool was used for chamber installation which prepared slots for the chamber to be pushed approximately 5 cm into the soil. To ensure a good seal between the chamber and the soil it was essential that all sides of the chamber were fully inserted, so this was checked thoroughly. On each sampling date lids were placed on the chambers at time 0. Following this, 60 ml samples were taken from the chamber headspace using a plastic syringe after 0, 30 and 60 min. The samples were flushed through pre-evacuated, airtight, 20 ml vials using a needle. In between sampling dates, the lids were removed from the chambers in order to re-expose the soil and vegetation inside the chamber to ambient conditions of light and rainfall. Fluxes were calculated based on the rate of change in gas concentration inside the chamber after 30 min for  $\text{CO}_2$  and  $\text{CH}_4$  and 60 min for  $\text{N}_2\text{O}$ . Accumulation of the gases was shown to be linear during these closed periods during a previous trial (data not shown) and a linear increase was assumed when calculating fluxes from all chambers.

Subsurface gas samplers were based on the design of Clark et al. (2001). The key component is a 10 cm length of gas permeable silicone rubber tubing (11.5 mm diameter Tygon<sup>®</sup> 3350 sanitary tubing). Jacinthe and Dick (1996) and DeSutter et al. (2006) showed that an equilibration period of less than 6 h is required for the target gases to closely match soil atmospheric concentrations in an unflooded soil. The body of the sampler was a 60 ml syringe which served as a headspace container ( $140 \text{ mm} \times 25 \text{ mm}$ ) with a septum to allow manual needle sampling. The connection between the syringe unit and the silicone rubber tube was a length of gas impermeable, flexible Tygon<sup>®</sup> fuel and lubricant tubing allowing a horizontal alignment of the silicone tubing at a single depth, rather than a profile (vertical) alignment (Clark et al., 2001). All connections within the unit were sealed with bungs and silicone sealant to ensure gas and water-tight seals.

The samplers were installed two weeks prior to the commencement of the experiment. A 35 cm trench was dug and the soil and vegetation carefully removed. A tool consisting of a long handle and a pointed extrusion to the diameter of the Tygon tubing was inserted into intact soil at the side of the trench at 10 cm, 20 cm and 30 cm depth and then the sampler tubes inserted. The displaced soil and vegetation was then carefully replaced around the sampler, staying as close as possible to the original layering and bulk density. Vegetation regrew around the samplers within the space of one month. On each sampling date a single 30 ml sample was taken from each sampler and flushed through a 20 ml pre-evacuated vial



for storage and transport. Immediately following sampling, ambient air was allowed back into the sampler to regain equal pressure between the sampler and the atmosphere.

All gas samples were analysed using a PerkinElmer Clarus 500 gas chromatograph (GC) with a Flame Ionisation Detector (FID) at 350 °C for CO<sub>2</sub> and CH<sub>4</sub> detection and a <sup>63</sup>Ni Electron Capture Detector (ECD) at 300 °C for the detection of N<sub>2</sub>O. A Turbo Matrix 110 auto-sampler extracted a 0.03 µL min<sup>-1</sup> sample from each vial and injected it through two 30m × 0.53 mm Elite Plot Q columns. The GC system had a minimum detectable amount (MDA) of 0.33, 0.15 and 0.004 ppm for CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O respectively. Samples were analysed within two weeks of collection.

#### 2.4. Soil sampling and analysis

Two soil cores were taken from each plot on each sampling date using a 5 cm × 15 cm Dutch auger. These were split into three depths (0–10 cm, 10–20 cm and 20–30 cm) and bulked together by depth within each plot, therefore 30 samples were collected on each occasion providing five replicates of each depth for each treatment. Upon return to the laboratory soil samples were stored at 4 °C prior to analysis (within one week of their collection).

Soil was sieved to 4 mm to remove roots and other debris. A 50 g subsample was weighed and then placed in an oven overnight at 105 °C for gravimetric moisture determination. Soil was then analysed for NH<sub>4</sub><sup>+</sup>-N and Total Oxidised Nitrogen (TON) using the KCl extraction technique (Bremner and Keeney, 1966). TON is the sum of NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> and for the purposes of this study is assumed approximately equivalent to NO<sub>3</sub><sup>-</sup> content as NO<sub>2</sub><sup>-</sup> is generally short-lived in the soil and accumulation is negligible. All analyses for TON and NH<sub>4</sub><sup>+</sup> were performed using an Aquakem 250 or Skalar 5000-02 Autoanalyser.

Soil was analysed for water soluble (available) carbon (WSOC) using a cold water extraction technique with a ratio of one part soil to five parts deionised water agitated for 2 h in an orbital shaker (e.g. McGill et al., 1986; Lu et al., 2011). WSOC analysis was performed using a Skalar Formacs<sup>HT</sup> TOC Analyser. WSOC was defined as the difference between total C and inorganic C in the solution.

#### 2.5. Statistical analyses

All statistical processing was carried out using Genstat 13th edition (2010). Student's *t*-tests were used to compare greenhouse gas fluxes and subsurface concentrations and soil NH<sub>4</sub><sup>+</sup>, TON and WSOC concentrations between treated and control plots. For each soil depth (0–10 cm, 10–20 cm and 20–30 cm), the significance of the treatment over time on subsurface greenhouse gas concentrations, NH<sub>4</sub><sup>+</sup>, TON and WSOC was assessed using a two-way repeated measures ANOVA where the degrees of freedom for the *F*-test were scaled by the Greenhouse–Geisser epsilon coefficient.

Daily CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O fluxes were calculated on each measurement occasion. The area under the curve (trapezoidal) method (e.g. Cardenas et al., 2010) was used to calculate cumulative fluxes for each gas across the entire sampling period. These were calculated from the adjusted predictions from general linear regression models of date and location. The proportion of the added N that was emitted from the soil surface was calculated using the average cumulative flux from control plots subtracted from the average cumulative flux from treated plots. The trace N content of the ditch-water (control) applications was assumed to be negligible.

The attribution of factors to GHG fluxes or concentrations was achieved using multiple regression models. Forward selection all-subsets regression was used in the first instance to identify contributing factors to GHG production at each depth and to emissions from the surface. WSOC, NH<sub>4</sub><sup>+</sup>, TON, WTD and ambient temperature were included as factors. Although time (days after application) was

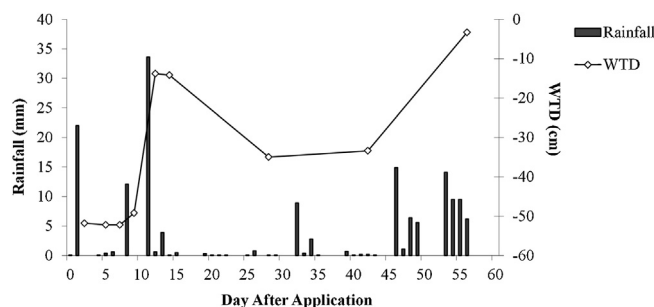


Fig. 1. Rainfall (mm) and average water-table depth (cm ± SE, N = 5) throughout the experimental period.

initially also included as a factor, analysis showed that it followed the same trends as WTD therefore the decision was taken to remove it from the regression models. Following the identification of the most significant contributing factors, a stepwise generalised linear regression was used to fit these terms.

### 3. Results

#### 3.1. Environmental Variables

During the experimental period, maximum air temperatures fluctuated between 8 and 22 °C and minimum temperatures between -3 and 10 °C with a general downward trend. The minimum temperature dropped to below freezing overnight on occasions. There were several episodes of rainfall throughout the experiment, notably rainfall exceeded 10 mm on Days 1, 8, 11, 32 and from Day 46 (after urine application) onwards (Fig. 1). For the first week after application the water-table was steady, at approximately 55 cm depth below the ground surface. Following the first week the water-table fluctuated in response to rainfall (Fig. 1). By the final sampling date 56 days after urine application, the water-table had risen to approximately 3 cm below the surface. Gravimetric soil moisture varied between 61 and 81% with the majority of the variation as a result of the profile depth of soil sampling ( $P < 0.001$ ) and no significance as a result of date ( $P = 0.985$ ) or treatment ( $P = 0.926$ ). This is likely to be due to the soil type; peat is known to retain moisture due to the high organic matter content and there were incidences of rainfall and decreases in WTD after the first week of the experiment (Fig. 1). The gravimetric methodology does not allow finely accurate quantifications of soil moisture and therefore WTD is considered to be a stronger indicator of soil hydrological conditions than measured water content for the purposes of this study. An in-situ dielectric soil moisture probe calibrated specifically for organic soils may provide the depth of information required to use soil moisture as an explanatory variable.

Soil NH<sub>4</sub><sup>+</sup>, TON and WSOC in the 0–10 cm surface layer (the layer showing the most substantial temporal and treatment variation throughout the experimental period) are summarised in Table 2.

Soil NH<sub>4</sub><sup>+</sup> concentrations were between 15 and 300 mg N kg dry soil<sup>-1</sup> throughout the sampling period (Table 2). Lower concentrations (0–50 mg N kg dry soil<sup>-1</sup>) were observed at 10–30 cm soil depth. Soil NH<sub>4</sub><sup>+</sup> concentrations were significantly higher ( $P < 0.001$ ) in the plots treated with cattle urine than in the control plots at 0–10 cm and 10–20 cm soil depth but not significant ( $P = 0.165$ ) at 20–30 cm soil depth. In the urine treated plots, NH<sub>4</sub><sup>+</sup> concentrations at 0–10 cm depth increased substantially between 12 and 28 and again between 28 and 42 days after urine application (Table 2). There was a significant effect of sampling date on soil NH<sub>4</sub><sup>+</sup> concentrations at 0–10 cm ( $P = 0.019$ ) but not at 10–20 cm or 20–30 cm. NH<sub>4</sub><sup>+</sup> concentrations in the control soil remained below 20 mg N kg dry soil<sup>-1</sup> with a generally decreasing trend towards the end of the experimental period.

Soil TON concentrations peaked at 284 mg N kg dry soil<sup>-1</sup> on Day 12 in the 0–10 cm soil layer of the treated plots and decreased from this point forward (Table 2). As for NH<sub>4</sub><sup>+</sup>, lower concentrations (0–150 mg N kg dry soil<sup>-1</sup>) were observed at 10 to 30 cm soil depth. There was a significant effect of the cattle urine treatment at all depths ( $P < 0.001$  in all cases). There was no significance of sampling date at 0–10 cm ( $P = 0.165$ ) but there was evidence of significant temporal variation at 10–20 and 20–30 cm depth. Until the final sampling date, TON concentrations were always significantly higher at 0–10 cm than at 10–20 or 20–30 cm depths in urine treated plots. TON concentration in the control soil remained below 30 mg N kg dry soil<sup>-1</sup> throughout the experimental period.

Soil WSOC in both urine treated and control plots were within the range of 29–50 mg C kg dry soil<sup>-1</sup> at 0–10 cm soil depth (Table 2) and 10–20 cm soil depth. WSOC concentrations were consistently higher at 20–30 cm soil depth, but were within the range 30–70 mg C kg dry soil<sup>-1</sup> at 20–30 cm due to the higher organic matter content of the peat layer. For all sampled depths, WSOC concentrations were not significantly different between urine treated and control plots ( $P = 0.121, 0.373$  and 0.222 for 0–10, 10–20 and 20–30 cm respectively). There was a significant effect

**Table 2**

Average TON,  $\text{NH}_4^+$  and WSOC at 0–10 cm soil depth on selected dates throughout the experimental period in treated (cattle urine) and control (ditch water) plots ( $\pm$  standard error).  $N=5$ .

Day after application	Cattle urine treated			Ditch water treated		
	TON (mg N kg dry soil <sup>-1</sup> )	$\text{NH}_4^+$ (mg N kg dry soil <sup>-1</sup> )	WSOC (mg C kg dry soil <sup>-1</sup> )	TON (mg N kg dry soil <sup>-1</sup> )	$\text{NH}_4^+$ (mg N kg dry soil <sup>-1</sup> )	WSOC (mg C kg dry soil <sup>-1</sup> )
2	197.4 $\pm$ 72.7	15.3 $\pm$ 1.1	32.3 $\pm$ 0.5	11.6 $\pm$ 1.2	9.6 $\pm$ 2.7	29.6 $\pm$ 0.6
5	172.4 $\pm$ 74.0	26.9 $\pm$ 4.3	36.3 $\pm$ 0.7	8.0 $\pm$ 1.6	19.2 $\pm$ 6.0	35.8 $\pm$ 0.6
12	284.5 $\pm$ 88.1	25.4 $\pm$ 5.3	35.5 $\pm$ 0.5	8.1 $\pm$ 0.7	13.4 $\pm$ 2.1	37.1 $\pm$ 0.9
28	185.2 $\pm$ 7.4	185.2 $\pm$ 50.7	41.2 $\pm$ 0.8	22.9 $\pm$ 2.1	3.2 $\pm$ 1.0	40.5 $\pm$ 0.5
42	68.5 $\pm$ 5.0	292.0 $\pm$ 93.7	32.2 $\pm$ 0.5	16.8 $\pm$ 1.5	0.0 $\pm$ 0.0	49.3 $\pm$ 0.6

of time after application on WSOC concentrations at all sampling depths ( $P=0.028$ , 0.002 and 0.002 for 0–10, 10–20 and 20–30 cm respectively).

### 3.2. Gaseous emissions

The  $\text{CO}_2$  fluxes peaked at  $5262 \text{ mg CO}_2 \text{ m}^{-2} \text{ d}^{-1}$  initially a few hours following urine application to the soil, exceeding baseline fluxes by approximately  $4000 \text{ mg CO}_2 \text{ m}^{-2} \text{ d}^{-1}$ . This peak was smaller in the control plots, suggesting that wetting of the soil alone did not prompt this  $\text{CO}_2$  release (Fig. 2a).  $\text{CO}_2$  fluxes were significantly higher from the urine treated plots than the control plots ( $P=0.010$ ) largely as a result of this substantial peak. A week after application,  $\text{CO}_2$  emissions from the urine treated plots followed the same trend as the control. Cumulative  $\text{CO}_2$  emissions over the full 56-day experimental period were higher from the urine treated plots than the control plots ( $42,014$  and  $29,462 \text{ mg CO}_2 \text{ m}^{-2}$  respectively).

The  $\text{CH}_4$  fluxes initially responded negatively to urine addition, with a mean negative flux after two days (Fig. 2b). There were no clear outliers in the treated chambers, with negative fluxes between  $-500$  and  $-3000 \mu\text{g CH}_4 \text{ m}^{-2} \text{ d}^{-1}$  for all chambers; however, for the control plots there were three chambers within the range of  $-2000$  to  $-3400 \mu\text{g CH}_4 \text{ m}^{-2} \text{ d}^{-1}$  and two giving positive fluxes of  $186$  and  $3649 \mu\text{g CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ . Following this date,  $\text{CH}_4$  from the urine treated plots was consistently higher than from the control plots with a peak (up to  $19 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ ) evident on Day 12. There was no significant difference between  $\text{CH}_4$  fluxes from treated and control plots ( $P=0.111$ ). Despite the early  $\text{CH}_4$  uptake flux, cumulative  $\text{CH}_4$  emissions showed net emission from the urine treated plots, whereas the control plots remained an overall  $\text{CH}_4$  sink ( $540$  and  $-13,696 \mu\text{g CH}_4 \text{ m}^{-2}$  from the treated and control plots respectively).

Two peaks of  $\text{N}_2\text{O}$  emission were observed during the experimental period (Fig. 2c). The first ( $20 \text{ mg N}_2\text{O m}^{-2} \text{ d}^{-1}$ ) was on Day 7 and the second and most pronounced peak (up to  $88 \text{ mg N}_2\text{O m}^{-2} \text{ d}^{-1}$ ) was measured on Day 12 following heavy rain (Fig. 1). Emissions from treated plots were always significantly higher than the control ( $P<0.001$ ) and had not returned to background levels by the end of the study. Cumulative emissions showed the clear increase in  $\text{N}_2\text{O}$  fluxes following urine application as total emissions from the control site were several orders of

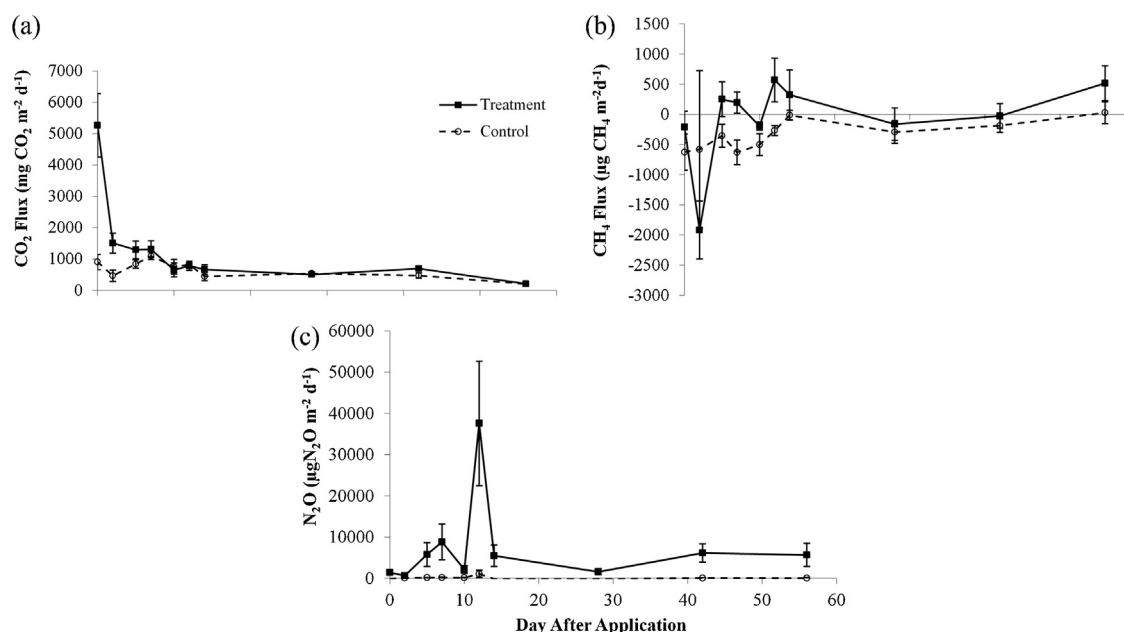
magnitude lower ( $326 \text{ mg N}_2\text{O m}^{-2}$  and  $916 \mu\text{g N}_2\text{O m}^{-2}$  from the treated and control plots respectively). Over the study period, cumulative emissions from the urine treated plots were 356 times higher than those from the control plots. The total emitted  $\text{N}_2\text{O}$  during the 8-week measurement period (urine treated minus control) represented 0.65% of the added N from the urine.

### 3.3. Subsurface gas concentrations

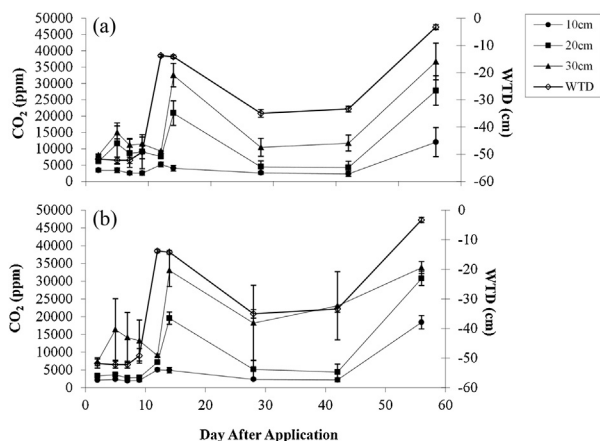
Concentrations of  $\text{CO}_2$  in the subsurface samplers were within a similar range for treated and control plots with clear increases by depth (Fig. 3). There was no significant difference in subsurface  $\text{CO}_2$  concentrations between treated and control plots for any depth. There was a significant effect of date after application ( $P<0.001$  at all depths). In both urine treated and control plots,  $\text{CO}_2$  concentrations at depth increased as the water-table moved towards the surface. The highest  $\text{CO}_2$  concentrations were measured 14 and 56 days after application, corresponding to shallow WTD (Fig. 1).

Subsurface  $\text{CH}_4$  concentration was significantly higher in the urine treated plots than in the control plots at 20–30 cm ( $P=0.010$ ) but not at the shallower soil depths (Fig. 4). Day after application was a significant driver of variation in  $\text{CH}_4$  concentrations at 0–10 ( $P<0.001$ ) and 10–20 cm depth ( $P=0.005$ ) but not at 20–30 cm ( $P=0.064$ ). This suggests that the  $\text{CH}_4$  concentrations in all plots were more subject to relatively natural variations such as water-table change than the treatment.

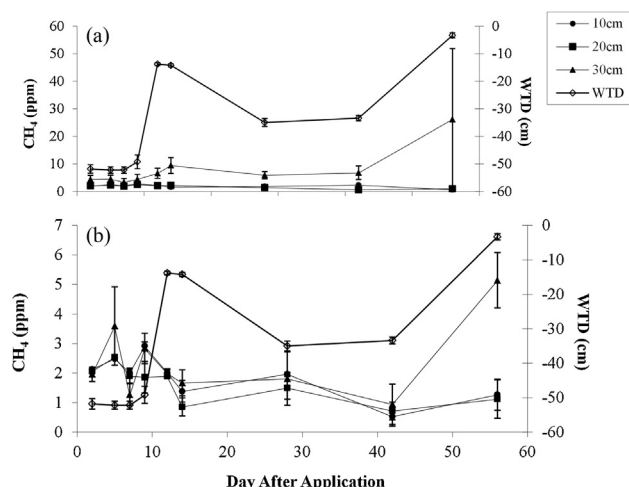
Average subsurface concentrations of  $\text{N}_2\text{O}$  were significantly higher in the urine treated plots than in the control plots at all soil depths ( $P=0.005$  for 0–10 cm, 0.002 for 10–20 cm and 0.034 at 20–30 cm, Fig. 5). Day after application was a significant factor controlling subsurface  $\text{N}_2\text{O}$  concentrations in the surface 20 cm ( $P=0.017$  for 0–10 cm and  $P<0.001$  for 10–20 cm) but not significant at 20–30 cm soil depth. Variation of  $\text{N}_2\text{O}$  over time for both control and treated plots tracked WTD variation, especially at 20 cm soil depth (Fig. 5). As for  $\text{CO}_2$ , soil  $\text{N}_2\text{O}$  concentration peaked on Day 14 and Day 56 for the urine treated plots (Fig. 5a) and this corresponded with shallower WTD (Fig. 1). By the second day following urine addition, there was already a significant difference between control and treated plots for all depths (Fig. 5a). On Day 12, there was an increase in  $\text{N}_2\text{O}$  concentration at 20 cm depth of



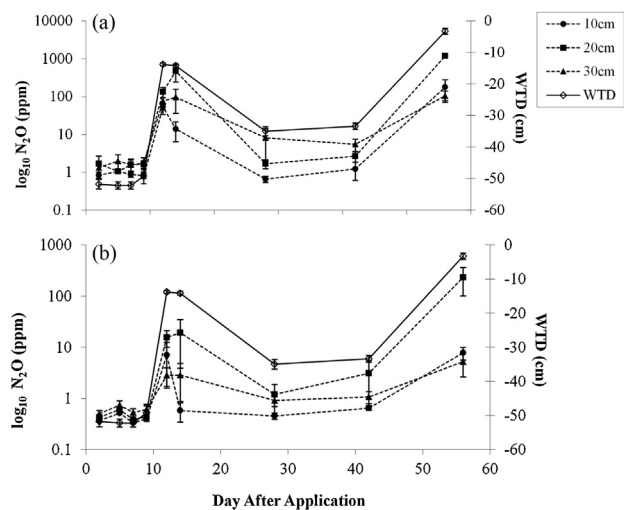
**Fig. 2.** Average flux of (a)  $\text{CO}_2$ , (b)  $\text{CH}_4$  and (c)  $\text{N}_2\text{O}$  for urine-amended and ditch water amended control plots by day after treatment application. Error bars reflect  $\pm$  standard error of the mean.  $N=5$ .



**Fig. 3.** Average subsurface CO<sub>2</sub> concentrations in (a) the urine treated plots and (b) the ditch water amended control plots. Error bars reflect  $\pm$  standard error of the mean.  $N=5$ .



**Fig. 4.** Average subsurface CH<sub>4</sub> concentrations in (a) the urine treated plots and (b) the ditch water amended control plots. Error bars reflect  $\pm$  standard error of the mean.  $N=5$ .



**Fig. 5.** Average subsurface log<sub>10</sub> N<sub>2</sub>O concentrations in (a) the urine treated plots and (b) the ditch water amended control plots. Error bars reflect  $\pm$  standard error of the mean.  $N=5$ .

over 30 times the level recorded on in the urine treated plots and the control plot (Fig. 5a and b). By Day 14 the subsurface N<sub>2</sub>O in control plots had increased by a factor of up to 4.5 at 20 cm, although there was a concentration decrease at 10 cm in the urine treated plots (Fig. 5a). On the final sampling day, there was another large rise in production at 20 cm in both the urine treated and control plots, with some values at over double those recorded on Day 14. For both urine treated and control plots the highest N<sub>2</sub>O concentrations were detected at 20 cm depth during these peak production events.

### 3.4. Controls on GHG fluxes and subsurface concentrations

Regression analysis indicated WTD was the key control on CO<sub>2</sub> flux, although this explained only 12% of variation. WSOC at any depth contributed a negligible improvement to the model fit. WTD was also the only significant control on CO<sub>2</sub> concentrations at depth, explaining 24.0%, 32.5% and 14.6% of variation in CO<sub>2</sub> at 10 cm, 20 cm and 30 cm depth respectively.

Variation in CH<sub>4</sub> fluxes was controlled by WTD and WSOC measured at 10–20 cm soil depth; however, the variation explained by this model was very low (6.2%). These results suggested that no measured variables were significant controlling factors on CH<sub>4</sub> emission from this site. Subsurface CH<sub>4</sub> concentrations were explained by combinations of WTD, WSOC and NH<sub>4</sub><sup>+</sup>. WTD and NH<sub>4</sub><sup>+</sup> in the surface 20 cm of soil explained the greater part of the variation of CH<sub>4</sub> concentrations at 10 cm depth (44.2%). Stepwise linear regression for the identified terms and CH<sub>4</sub> at 20 cm showed WTD accounted for 9.7% of the variation. Addition of NH<sub>4</sub><sup>+</sup> measured in soil taken from 0–10 cm to 10–20 cm depth improved the model fit to 24.0%. The WSOC measured at 0–10 cm provided small increases to the model fit. The CH<sub>4</sub> concentrations at 30 cm were only explained by variation in NH<sub>4</sub><sup>+</sup> in the surface 20 cm of soil (48.2% of variance explained). Inclusion of WTD did not improve the model.

The N<sub>2</sub>O fluxes were explained by WTD and surface TON and NH<sub>4</sub><sup>+</sup>. WTD alone accounted for 21.0% of the variation. Adding NO<sub>3</sub><sup>−</sup> content at 0–10 cm improved this to 26.6%. Therefore WTD, followed by TON at 0–10 cm, followed by NH<sub>4</sub><sup>+</sup> at 0–10 cm was the order of importance of these controlling factors. Subsurface N<sub>2</sub>O concentrations were explained by WTD, WSOC and TON. Addition of NH<sub>4</sub><sup>+</sup> did not improve the fit of models of subsurface N<sub>2</sub>O concentrations.

## 4. Discussion

### 4.1. Effect of urine addition on soil NH<sub>4</sub><sup>+</sup>, TON and WSOC

Addition of cattle urine increased concentrations of NH<sub>4</sub><sup>+</sup> and TON in the soil relative to a control. There was a substantial increase in NH<sub>4</sub><sup>+</sup> in the 0–10 cm soil layer between 12 and 28 days after urine application and further increases to the end of the experimental period. A smaller increase was observed in the 10–20 cm soil layer and little increase was observed at 20–30 cm. This accumulation of NH<sub>4</sub><sup>+</sup> in the surface layer may be due to mineralisation of the urine (Allen et al., 1996), suggested also by the decrease in WSOC between days 28 and 42 in the treated plots (but not the controls); however, this is difficult to confirm with low temporal resolution data as changes in WSOC between 12 and 28 days corresponding to mineralisation of organic carbon could not be detected. However, the key control of the observed accumulation is likely to be the sustained shallow WTD during this period. Although there was a variation of around 20 cm, the water-table was observed to remain around 30 cm below the surface and soil moisture was likely to be maintained due to rainfall (Fig. 1). This would have maintained anoxic conditions that are not well suited to nitrification; a mechanism that may have been preventing significant accumulation of NH<sub>4</sub><sup>+</sup>. Accumulation of NH<sub>4</sub><sup>+</sup> is known to be an indicator of denitrification because reduced WTD creates anoxic conditions better suited to denitrification processes than nitrification processes (Nieder and Benbi, 2008) and has previously been shown to be higher and more variable in peat and clay soils than sandy soils after urine addition (Clough et al., 1998).

Soil TON (approximately equal to NO<sub>3</sub><sup>−</sup> concentrations as NO<sub>2</sub><sup>−</sup> was anticipated to be limited) concentration also peaked 12 days after application in the 0–10 cm soil layer but decreased in the surface 10 cm of soil from this point forward. This increase of TON during the first half of the study (corresponding to deep WTD) suggests that during this time nitrification was the key N<sub>2</sub>O producing process; however following this (corresponding to shallow WTD), there was a switch to denitrification. This is supported by



the increasing  $\text{NH}_4^+$  and decreasing TON. There was no evidence of leaching of  $\text{NO}_3^-$  to lower soil layers, suggesting either the low temporal resolution of soil sampling could not capture  $\text{NO}_3^-$  movement through the profile or there was substantial consumption of the  $\text{NO}_3^-$  in the topsoil. The overall consumption of  $\text{NO}_3^-$  in the West Sedgemoor topsoil following urine application agrees with studies suggesting denitrification is the key N transformation process in peatland soils (e.g. Aerts and Ludwig, 1997; Nieder and Benbi, 2008); however, a large proportion of this  $\text{NO}_3^-$  may have been taken up by the vegetation (Urban et al., 1988). Similarly to this study, Li and Kelliher (2005) found that 2 months after urine application, the  $\text{NO}_3^-$  content in both soils remained greater than that of the controls, whereas Allen et al. (1996) found increased  $\text{NH}_4^+$  concentrations throughout a 70 day period after application but no change in  $\text{NO}_3^-$  concentration.

Addition of cattle urine did not have a significant effect on soil WSOC, suggesting substantial loss of the urine organic carbon pool through hydrolysis within the first two days after application (Li and Kelliher, 2007; Lin et al., 2009). Very similar variation in WSOC over time was shown in both treated and control soils. This is similar to the observations of Kelliher et al. (2005) who showed an increase in soil carbon at 0–10 cm immediately following urine application to soil samples from a dairy farm which began to fall to background levels by two days after application. However, they also found that following urine addition to samples from an ungrazed grassland soil, WSOC in the topsoil remained elevated for eleven days. In their study, no vegetation was present which may account for the rapid loss of the available carbon pool in the West Sedgemoor field shortly after application. There was no evidence of increased WSOC in the treated plots and therefore the remaining C was probably rapidly leached from the top 30 cm of soil or taken up by vegetation. For future work, isotopic labelling of urine C may be used to accurately determine the movement of C through the soil following application to determine the fate of added C in peat soil (Bol et al., 2004; Lambie et al., 2012, 2013). A higher frequency of soil sampling would also be useful in future studies to examine the rate of the loss of this pool from the soil, particularly with reference to high resolution  $\text{CO}_2$  flux measurements.

#### 4.2. Nitrous oxide

Cumulative  $\text{N}_2\text{O}$  emission during this study was  $3.26 \text{ kg N}_2\text{O ha}^{-1}$  from the treated plots and emission from the control plots was negligible in comparison ( $0.009 \text{ kg N}_2\text{O ha}^{-1}$ ). Very low emission of  $\text{N}_2\text{O}$  was expected in this field as peatlands often have low amounts of soil N and there has not been substantial N addition to the field site for an extended period of time. This was supported by the low amounts of TON and  $\text{NH}_4^+$  consistently measured in the control plots throughout the experiment. The temporal variation in  $\text{N}_2\text{O}$  emission following cattle urine application found in this study is consistent with others in the literature (Koops et al., 1997; Anger et al., 2003; Di and Cameron, 2012). Li and Kelliher (2005), Maljanen et al. (2007) and Lin et al. (2009) found a peak in  $\text{N}_2\text{O}$  fluxes on the day of application, which was not detected at West Sedgemoor. Anger et al. (2003) suggested that a delay in  $\text{N}_2\text{O}$  emission following urine addition is due to an inactive nitrifier population on swards that do not receive regular N addition. On fertilised swards, they found more rapid and much greater initial  $\text{N}_2\text{O}$  release as a result of the nitrifier community having been primed for N addition. Delay in the emission of  $\text{NH}_4^+$  is likely to be due to a combination of gradual mineralisation of urea to  $\text{NH}_4^+$ , slow response of nitrifier communities to the  $\text{NH}_4^+$  increase and possibly competition with plants. The largest emissions were recorded on Day 12, following heavy rain and a rise in the water table by 20 cm. Rainfall has been widely shown to trigger substantial  $\text{N}_2\text{O}$  release following urine application (Allen

et al., 1996; Li and Kelliher, 2005; Di and Cameron, 2012). Research has shown that nitrification and denitrification can occur in soils simultaneously, particularly in short periods of high moisture, wherein nitrifying bacteria can turn to short-term denitrification (nitrifier denitrification) of  $\text{NO}_2^-$  to  $\text{N}_2$  via  $\text{N}_2\text{O}$  (Wrage et al., 2011). Regression analysis identified WTD and  $\text{NO}_3^-$  concentration at 0–10 cm as the key controls on surface flux although these did not explain a great deal of the variation (28.6%). This may be due to higher importance of other factors such as moisture content and inorganic N contents in the surface 1–2 cm of soil, as suggested by Neftel et al. (2007).

Cumulative  $\text{N}_2\text{O}$  emissions data showed increased fluxes with respect to the control plots. The total emitted  $\text{N}_2\text{O}$  (treated minus control) represented 0.65% of the added N from the urine. This figure lies within the expected ranges of values available from other short-term experimental studies (Li and Kelliher, 2005; Hoefel et al., 2012; Smith et al., 2012). Li and Kelliher (2005) found between 0.4 and 1.3% of added N was then emitted to the atmosphere over a 4-month period, with the higher values found in poorly drained soils. Total emitted  $\text{N}_2\text{O}$  appears to be higher for peat soils (between 2 and 4%), as a result of this anticipated waterlogging (Koops et al., 1997; van Beek et al., 2011).

The  $\text{N}_2\text{O}$  concentrations at depth initially corresponded to surface  $\text{N}_2\text{O}$  release, increasing substantially at 10 cm depth between Day 10 and Day 12. This increase was even more substantial at 20 cm depth with concentrations as high as 200 ppm in some plots. By Day 14  $\text{N}_2\text{O}$  production in the treated plots had increased yet further at 20 cm (values up to 1300 ppm) but had decreased at 10 cm and no peak in surface  $\text{N}_2\text{O}$  fluxes was detected.

The  $\text{N}_2\text{O}$  accumulation at 20 cm depth is likely to be a result of the physical changes in porosity and diffusion coefficient between soil horizons. Boon et al. (2013) showed a decrease in porosity and gaseous diffusion between the peat subsoil and the clay layer at this field site at lower airfilled porosities. The subsurface samplers were below or close to the water-table for much of the experimental period; therefore diffusion of the produced  $\text{N}_2\text{O}$  is likely to have been restricted at 20 cm, leading to the observed accumulation. The strong likelihood that the release of  $\text{N}_2\text{O}$  produced in the soil was controlled by the diffusion coefficient supports the findings of Balaine et al. (2013). Balaine et al. (2013) also showed that the production of  $\text{N}_2\text{O}$  is sensitive to changes in the diffusion coefficient and therefore finer scale monitoring or modelling of the diffusion coefficient in the surface soil may have aided explanation of  $\text{N}_2\text{O}$  production at 20 cm. There may also have been chemical changes between the peat and clay horizons which influenced  $\text{N}_2\text{O}$  production (Clough et al., 1998; Clough et al., 2003b); however, this study did not focus on variation in soil chemistry as a significant source of variation. Future work considering the combination of soil chemical variation and addition of cattle urine in peat soils may provide additional perspectives on the observed variability of  $\text{N}_2\text{O}$  production within the soil profile.

Although the highest  $\text{N}_2\text{O}$  concentrations were detected at 20 cm both for urine treated and control plots,  $\text{N}_2\text{O}$  emissions were only influenced strongly by changes detected at 10 cm. This is probably due to the low diffusion coefficient of  $\text{N}_2\text{O}$  through water ( $2.04 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ ; Grabbie, 1966) reducing its ability to pass through the waterlogged soil. In addition, denitrification of  $\text{N}_2\text{O}$  to  $\text{N}_2$  also depletes  $\text{N}_2\text{O}$  in an anaerobic soil (Terry et al., 1981; Arah et al., 1991). Maljanen et al. (2003) likewise suggested that when a soil is waterlogged only concentrations at 5 cm depth can be indicative of flux; however, when the soil is dry,  $\text{N}_2\text{O}$  at 20 cm correlated well with the surface flux. Neftel et al. (2007) state that  $\text{N}_2\text{O}$  fluxes are only influenced by the first 1–2 cm of soil, particularly where uptake fluxes are concerned; therefore surface fluxes cannot be easily predicted from  $\text{N}_2\text{O}$  concentrations below this depth. Surface measured  $\text{N}_2\text{O}$  fluxes are unlikely to be indicative of the

concentrations of  $\text{N}_2\text{O}$  at depth within the soil and likewise, fluxes calculated from subsurface concentrations may overestimate the amount of  $\text{N}_2\text{O}$  that actually reaches the surface.  $\text{N}_2\text{O}$  may also be carried from depth by mass movement events and episodic fluxes may occur that are not captured by low resolution studies.

Finally, ambient temperature was not shown to have a significant effect on greenhouse gas fluxes or subsurface concentrations by the regression analyses used to study these data. Air and soil temperature is known to have a positive correlation with greenhouse gas emissions due to stimulation of microbial metabolisms (Smith et al., 2003). The effect is complex for  $\text{N}_2\text{O}$  fluxes however, since temperature also controls the functionality of microorganisms facilitating  $\text{CH}_4$  oxidation and N volatilisation as  $\text{NH}_3$  gas (Lockyer and Whitehead, 1990; Sugimoto et al., 1993).

#### 4.3. Methane

The finding of an apparent increase in  $\text{CH}_4$  oxidation (negative fluxes) following application is contrary to expectation as most other studies found  $\text{CH}_4$  peaked shortly after application (Li and Kelliher, 2005; Lin et al., 2009) or did not influence on continual uptake fluxes (Liebig et al., 2008). Despite this negative peak, cumulative  $\text{CH}_4$  fluxes showed net emission of  $\text{CH}_4$  from the treatment plots compared with net uptake on the control plots. Regression modelling could not explain a high amount of variation in  $\text{CH}_4$  fluxes. Further study would be required to determine whether the negative fluxes of  $\text{CH}_4$  following urine application can be repeated and a higher temporal resolution would be beneficial to determine the duration of the negative response.

It has been shown that presence of  $\text{NH}_4^+$  can inhibit  $\text{CH}_4$  oxidising bacteria and promote  $\text{CH}_4$  production (Dobbie and Smith, 1996; Li and Kelliher, 2007; Lin et al., 2009), although quantifying this effect separately from the effect of water addition requires further research. This is the key mechanism that should increase  $\text{CH}_4$  emission from urine spots, simply by preventing its oxidation. However, the results of this experiment also suggest enhanced production of  $\text{CH}_4$ , or perhaps enhanced storage of  $\text{CH}_4$  at depth. However, closer examination of the data reveals a number of 'hotspots' that may be unrelated to the urine addition and rather associated with zones of anaerobicity in the soil (Blodau and Moore, 2003).  $\text{CH}_4$  concentrations of 0 (or close to 0) were much more frequent in the soil atmosphere samplers located in control plots than the urine treated plots, once more supporting the hypothesis that  $\text{NH}_4^+$  inhibition rather than enhanced  $\text{CH}_4$  production is the main cause of increased  $\text{CH}_4$  emission from the urine treated plots. The regression models for  $\text{CH}_4$  concentrations at depth also showed that  $\text{NH}_4^+$  was a significant control on  $\text{CH}_4$  concentrations; however, the nature of its influence varied between a positive and negative contribution. Further research at a higher temporal and spatial resolution, under a controlled environment would be useful to examine the importance of  $\text{NH}_4^+$  on subsurface  $\text{CH}_4$  concentrations in this soil.

#### 4.4. Carbon dioxide

The range of  $\text{CO}_2$  fluxes observed during this experiment supports findings of other studies on temperate peat soils (Carter et al., 2012; Danevčič et al., 2010; Maljanen et al., 2010), including an earlier study carried out at the same field site (Kechavarzi et al., 2007). The large peak in  $\text{CO}_2$  fluxes a few hours after urine application was also shown following yak urine application to a meadow soil in China (Lin et al., 2009). This is likely to be due to carbon release from hydrolysis of urea or promotion of microbial respiration (Kelliher et al., 2007; Lin et al., 2009). This may be related to the possible rapid loss of urine C from the topsoil discussed in Section 4.1. The  $\text{CO}_2$  peak was not evident in the control plots, suggesting that this was not a wetting effect. Lin et al. (2009) indicated that temperature was

the key control on  $\text{CO}_2$  emissions rather than soil WFPS, however the water table in their study field (an alpine environment) did not vary as substantially as found during the autumn rewetting at West Sedgemoor. Conversely, Uchida et al. (2011) found no temperature effect (varied between 11 and 23 °C) on the significant increase of  $\text{CO}_2$  following urine addition in a sub-tropical environment. Further research on controls on the effect of urine on  $\text{CO}_2$  emissions from temperate peatland soils would be beneficial in order to disentangle these effects. Subsurface  $\text{CO}_2$  concentrations recorded within the top 10 cm of soil would also be useful to determine the zones of production of this microbial response.

Cumulative emissions of  $\text{CO}_2$  showed that over the experimental period approximately 13 g of C was lost as  $\text{CO}_2$  during the experimental period (subtracting the cumulative  $\text{CO}_2$  from the control plots from that of the treated plots). This is 20% of the total added C, slightly higher than the 11% loss estimated by Bol et al. (2004) and 15% by Petersen et al. (2004), although these studies were shorter in duration. It is likely that there was not full capture of the  $\text{CO}_2$  loss from hydrolysis during the first day after application and therefore the cumulative emissions from the treated plots may be underestimated. There was little evidence of increased  $\text{CO}_2$  emission from the priming of soil C by the urine (Clough et al., 2003a; Lambie et al., 2013); however as discussed previously, a large proportion of urine-C may have been leached from the surface soil, leading to the limited change in WSOC observed in the surface soil layers.

Subsurface  $\text{CO}_2$  concentrations were within a similar range for treated and control plots and supported by the concentrations recorded in other studies on peatland soils (Jungkunst et al., 2008; Elberling et al., 2011). Regression models for subsurface concentrations of  $\text{CO}_2$  following urine addition showed WTD was the only measured factor that controlled variation for this gas. These results suggest no impact of cattle urine application on  $\text{CO}_2$  concentrations in the soil profile.

## 5. Conclusion

This study showed that there was a significant effect of cattle urine addition on both subsurface concentrations and emissions of  $\text{CH}_4$  and  $\text{N}_2\text{O}$  but urine addition had little long-term impact on  $\text{CO}_2$  fluxes. Cumulative emissions clearly showed the potential for considerable  $\text{N}_2\text{O}$  fluxes from this field site during periods of grazing. Regression analysis on the field data showed that only the inorganic N concentrations in the first 10 cm of soil have a significant relationship with the surface fluxes of  $\text{N}_2\text{O}$ . This analysis also identified water-table depth as the dominant control on  $\text{N}_2\text{O}$  production and emission. An accurate estimate of soil moisture would be beneficial in future studies to further examine the influence of soil hydrology on production and emission of greenhouse gases. Accumulation of  $\text{NH}_4^+$  and depletion of  $\text{NO}_3^-$  suggested denitrification as the major  $\text{N}_2\text{O}$  producing process. Regression analysis suggested  $\text{NH}_4^+$  to be a significant control of  $\text{CH}_4$  concentrations, supporting other studies that demonstrate the inhibitory effect of  $\text{NH}_4^+$  on methane oxidation. This research also found a significant increase in  $\text{CH}_4$  oxidation in the treated plots two days following application. Further research is required to understand the mechanisms behind this apparent initial increase in  $\text{CH}_4$  oxidation.

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## References

- Aerts, R., Ludwig, F., 1997. Water-table changes and nutritional status affect trace gas emissions from laboratory columns of peatland soils. *Soil Biol. Biochem.* 29, 1691–1698.
- Allen, A.G., Jarvis, S.C., Headon, D.M., 1996. Nitrous oxide emissions from soils due to inputs of nitrogen from excreta return by livestock on grazed grassland in the UK. *Soil Biol. Biochem.* 28, 597–607.
- Anger, M., Hoffman, C., Kühbauch, W., 2003. Nitrous oxide emission from artificial urine patches applied to different N-fertilized swards and estimated annual N<sub>2</sub>O emissions from differently fertilized pastures in an upland location in Germany. *Soil Use Man.* 19, 104–111.
- Arah, J.R.M., Smith, K.A., Crichton, A., Li, H.S., 1991. Nitrous oxide production and denitrification in Scottish arable soils. *J. Soil Sci.* 42, 351–367.
- Baggs, E.M., 2011. Soil microbial sources of nitrous oxide: recent advances in knowledge, emerging challenges and future direction. *Curr. Opin. Environ. Sustainabil.* 3, 321–327.
- Ball, B.C., 2013. Soil structure and greenhouse gas emissions: a synthesis of 20 years of experimentation. *Eur. J. Soil Sci.* 64, 357–373.
- Balaine, N., Clough, T.J., Beare, M.H., Thomas, S.M., Meenken, E.D., Ross, J.G., 2013. Changes in relative gas diffusivity explain soil nitrous oxide flux dynamics. *Soil Sci. Soc. Am. J.* 77, 1496–1505.
- Blodau, C., Moore, T.R., 2003. Micro scale CO<sub>2</sub> and CH<sub>4</sub> dynamics in a peat soil during a water fluctuation and sulfate pulse. *Soil Biol. Biochem.* 35, 535–547.
- Bol, R., Petersen, S.O., Christofides, C., Dittert, K., Hansen, M.N., 2004. Short-term N<sub>2</sub>O, CO<sub>2</sub>, NH<sub>3</sub> fluxes, and N/C transfers in a Danish grass-clover pasture after simulated urine deposition in autumn. *J. Plant Nutr. Soil Sci.* 167, 568–576.
- Boon, A., Robinson, J.S., Nightingale, P.D., Cardenas, L., Chadwick, D.R., Verhoef, A., 2013. Determination of the gas diffusion coefficient of a peat grassland soil. *Eur. J. Soil Sci.* 64, 681–687.
- Bristow, A.W., Whitehead, D.C., Cockburn, J.E., 1992. Nitrogenous constituents in the urine of cattle, sheep and goats. *J. Sci. Food Agric.* 59, 387–394.
- Bremner, J.M., Keeney, D.R., 1966. Determination and isotope-ratio analysis of different forms of nitrogen in soils 3: exchangeable ammonium, nitrate and nitrite by extraction-distillation methods. *Proc. Soil Sci. Soc. Am.* 30, 577–582.
- Cardenas, L.M., Thorman, R., Ashlee, N., Butler, M., Chadwick, D., Chambers, B., 2010. Quantifying annual N<sub>2</sub>O emission fluxes from grazed grassland under a range of inorganic fertiliser nitrogen inputs. *Agric. Ecosyst. Environ.* 136, 218–226.
- Carter, M.S., Larsen, K.S., Emmett, B., Estiarte, M., Field, C., Leith, I.D., Lund, M., Meijde, A., Mills, R.T.E., Niinemets, Ü., Peñuelas, J., Portillo-Estrada, M., Schmidt, I.K., Selsted, M.B., Sheppard, L.J., Sowerby, A., Tietema, A., Beier, C., 2012. Synthesizing greenhouse gas fluxes across nine European peatlands and shrublands – responses to climatic and environmental changes. *Biogeosciences* 9, 3739–3755.
- Clark, M., Jarvis, S., Maltby, E., 2001. An improved technique for measuring concentration of soil gases at depth *in situ*. *Comm. Soil Sci. Plant Anal.* 32, 369–377.
- Clough, T.J., Ledgard, S.F., Sprosen, M.S., Kear, M.J., 1998. Fate of N-15 labelled urine on four soil types. *Plant Soil* 199, 195–203.
- Clough, T.J., Sherlock, R.R., Kelliher, F.M., 2003a. Can liming mitigate N<sub>2</sub>O fluxes from a urine-amended soil? *Aust. J. Soil Res.* 41, 439–457.
- Clough, T.J., Sherlock, R.R., Mautner, M.N., Milligan, D.B., Wilson, P.F., Freeman, C.G., McEwan, M.J., 2003b. Emission of nitrogen oxides and ammonia from varying rates of applied synthetic urine and correlations with soil chemistry. *Aust. J. Soil Res.* 41, 421–438.
- Danevčič, T., Mandić-Mulec, I., Stres, B., Stopar, D., Hacin, J., 2010. Emissions of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O from Southern European peatlands. *Soil Biol. Biochem.* 42, 1437–1446.
- DeSutter, T.M., Sauer, T.J., Parkin, T.B., 2006. Porous tubing for use in monitoring soil CO<sub>2</sub> concentrations. *Soil Biol. Biochem.* 38, 2676–2681.
- Di, H.J., Cameron, K.C., 2002. Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. *Nutr. Cyc. Agroecosys.* 64, 237–256.
- Di, H.J., Cameron, K.C., 2012. How does the application of different nitrification inhibitors affect nitrous oxide emissions and nitrate leaching from cow urine in grazed pastures? *Soil Use Manage.* 28, 54–61.
- Dobbie, K.E., Smith, K.A., 1996. Comparison of CH<sub>4</sub> oxidation rates in woodland, arable and set aside soils. *Soil Biol. Biochem.* 28, 1357–1365.
- Elberling, B., Askaer, L., Jørgensen, C.J., Joensen, H.P., Kühl, M., Glud, R.N., Lauritsen, F.R., 2011. Linking soil O<sub>2</sub>, CO<sub>2</sub>, and CH<sub>4</sub> concentrations in a wetland soil: implications for CO<sub>2</sub> and CH<sub>4</sub> fluxes. *Environ. Sci. Technol.* 45, 3393–3399.
- Findlay, D.C., Colborne, G.J.N., Cope, D.W., Harrod, T.R., Hogan, D.V., Staines, S.J., 1984. Soils and Their Use in South West England. Soil Survey of England and Wales Bulletin No. 14. Lawes Agricultural Trust (Soil Survey of England and Wales), Rothamsted Experimental Station, Harpenden.
- Firestone, M.K., Firestone, R.B., Tiedje, J.M., 1980. Nitrous oxide from soil denitrification. Factors controlling its biological production. *Science* 208, 749–751.
- Forster, P., Ramaswamy, V., Artaxo, P., Bernsten, T., Betts, R., Fahey, D.W., Haywood, J., Lean, J., Lowe, D.C., Myhre, G., Nganga, J., Prinn, R., Raga, G., Schulz, M., Van Dorland, R., 2007. Chapter 2. Changes in Atmospheric Constituents and in Radiative Forcing. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), *Climate Change 2007: The Physical Science Basis*. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom/New York, NY, USA.
- Grabbe, A.R., 1966. *Soil aeration and plant growth*. Adv. Agron. 18, 57–106.
- Haynes, R.J., Williams, P.H., 1993. Nutrient cycling and soil fertility in the grazed pasture ecosystem. Adv. Agron. 49, 119–199.
- Heathwaite, A.L., Ross, S.M., 1987. Evaluation of qualitative and quantitative classifications for fen peat in the Somerset levels, England. *J. Biogeogr.* 14 (2), 129–143.
- Hoef, I., Steude, K., Wrage, N., Veldkamp, E., 2012. Response of nitrogen oxide emissions to grazer species and plant species composition in temperate agricultural grassland. *Agric. Ecosyst. Environ.* 151, 34–43.
- Hutchinson, G.L., Livingston, G.P., 2002. Gas flux. In: Dane, J.H., Topp, G.C. (Eds.), *Methods of Soil Analysis, Part 1. Physical Methods*, 3rd edition. Soil Science Society of America, Madison, WI, pp. 1159–1182.
- IPCC, 2001. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. In: Houghton, J.T., Ding, Y., Griggs, D.J., Noguer, M., van der Linden, P.J., Dai, X., Maskell, K., Johnson, C.A. (Eds.), *Climate Change 2001: The Scientific Basis*. Cambridge University Press, Cambridge.
- Jacinto, P.A., Dick, W.A., 1996. Use of silicone tubing to sample nitrous oxide in the soil atmosphere. *Soil Biol. Biochem.* 28, 721–726.
- Jungkunst, H.F., Flessa, H., Scherber, C., Fiedler, S., 2008. Groundwater level controls CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub> fluxes of three different hydromorphic soil types of a temperate forest ecosystem. *Soil Biol. Biochem.* 40, 2047–2054.
- Kechavarzi, C., Dawson, Q., Leeds-Harrison, P.B., Szatylowicz, J., Gnatowski, T., 2007. Water-table management in lowland UK peat soils and its potential impact on CO<sub>2</sub> emission. *Soil Use Manage.* 23, 359–367.
- Kechavarzi, C., Dawson, Q., Leeds-Harrison, P.B., 2010. Physical properties of low-lying agricultural peat soils in England. *Geoderma* 154, 196–202.
- Kelliher, F.M., Sedcole, J.R., Minchin, R.F., Wan, Y., Condron, L.M., Clough, T.J., Bol, R., 2005. Soil microbial respiration responses to repeated urea applications in grasslands. *Aust. J. Soil Res.* 43, 905–913.
- Kelliher, F.M., Sedcole, J.R., Emery, I., Condron, L.M., 2007. Grassland soil microbial respiration responses to urea and litter applications. *N. Z. J. Agric. Res.* 50, 321–326.
- Koops, J.G., van Beusichem, M.L., Oenema, O., 1997. Nitrous oxide production, its source and distribution in urine patches on grassland on peat soil. *Plant Soil* 191, 57–65.
- Lambie, S.M., Schipper, L.A., Balks, M.R., Baisden, W.T., 2012. Carbon leaching from undisturbed soil cores treated with dairy cow urine. *Soil Res.* 50, 320–327.
- Lambie, S.M., Schipper, L.A., Balks, M.R., Baisden, W.T., 2013. Priming of soil decomposition leads to losses of carbon in soil treated with cow urine. *Soil Res.* 51, 513–520.
- Leterme, P., Barre, C., Vertes, F., 2003. The fate of <sup>15</sup>N from dairy cow urine under pasture receiving different rates of N fertilizer. *Agronomie* 23, 609–616.
- Li, Z., Kelliher, F.M., 2005. Determining nitrous oxide emissions from subsurface measurements in grazed pasture: a field trial of alternative technology. *Aust. J. Soil Res.* 43, 677–687.
- Li, Z., Kelliher, F.M., 2007. Methane oxidation in freely and poorly drained grassland soils and effects of cattle urine application. *J. Environ. Qual.* 36, 1241–1248.
- Liebig, M.A., Kronberg, S.L., Gross, J.R., 2008. Effects of normal and altered cattle urine on short-term greenhouse gas flux from mixed-grass prairie in the Northern Great Plain. *Agric. Ecosyst. Environ.* 125, 57–64.
- Lin, X., Wang, S., Ma, X., Xu, G., Luo, C., Li, Y., Jiang, G., Xie, Z., 2009. Fluxes of CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O in an alpine meadow affected by yak excreta on the Qinghai-Tibetan plateau during summer grazing periods. *Soil Biol. Biochem.* 41, 718–725.
- Lockyer, D.R., Whitehead, D.C., 1990. Volatilization of ammonia from cattle urine applied to grassland. *Soil Biol. Biochem.* 22, 1137–1142.
- Lu, X., Fan, J., Yan, Y., Wang, X., 2011. Soil water soluble organic carbon under three alpine grassland types in Northern Tibet, China. *Afr. J. Agric. Res.* 6, 2066–2071.
- Maljanen, M., Liikanen, A., Silvola, J., Martikainen, P.J., 2003. Measuring N<sub>2</sub>O emissions from organic soils by closed chamber or soil/snow gradient methods. *Eur. J. Soil Sci.* 54, 625–631.
- Maljanen, M., Martikainen, M., Koponen, H.T., Virkajärvi, P., Martikainen, P.J., 2007. Fluxes of nitrous oxide and nitric oxide from experimental excreta patches in boreal agricultural soil. *Soil Biol. Biochem.* 39, 914–920.
- Maljanen, M., Sigurdsson, B.D., Gumundsson, J., Óskarsson, H., Huttunen, J.T., Martikainen, P.J., 2010. Greenhouse gas balances of managed peatlands in the Nordic countries – present knowledge and gaps. *Biogeosciences* 7, 2711–2738.
- McGill, W.B., Cannon, K.R., Robertson, J.A., Cook, F.D., 1986. Dynamics of soil microbial biomass and water-soluble organic C in Breton I after 50 years of cropping to two rotations. *Can. J. Soil Sci.* 66, 1–19.
- Moir, J.L., Cameron, K.C., Di, H.J., Ferstak, U., 2011. The spatial coverage of dairy cattle urine patches in an intensively grazed pasture system. *J. Agric. Sci.* 149, 473–485.
- Moore, T.R., Dalva, M., 1993. The influence of temperature and water-table position on carbon dioxide and methane emissions from laboratory columns of peatland soils. *J. Soil Sci.* 44, 651–664.
- Mosier, A.R., 1989. Chamber and isotope techniques. In: Andreae, M.O., Schimel, D.S. (Eds.), *Exchange of Trace Gases between Terrestrial Ecosystems and the Atmosphere*, Life Sciences Research Report 47. John Wiley, Chichester, pp. 175–187.
- Mosier, A., Schimel, D., Valentine, D., Bronson, K., Parton, W., 1991. Methane and nitrous oxide fluxes in native, fertilized and cultivated grasslands. *Nature* 350, 330–332.
- Nieder, R., Benbi, D.K., 2008. *Bidirectional Biosphere–Atmosphere Interactions. Carbon and Nitrogen in the Terrestrial Environment*. Springer Science + Business Media B.V.

- Neftel, A., Fletchard, C., Ammann, A., Conen, F., Emmenegger, L., Zeyer, K., 2007. Experimental assessment of  $N_2O$  background fluxes in grassland systems. *Tellus* 59B, 470–482.
- Oenema, O., Velthof, G.L., Yamulki, S., Jarvis, S.C., 1997. Nitrous oxide emissions from grazed grassland. *Soil Use Manage.* 13, 288–295.
- Petersen, S.O., Roslev, P., Bol, R., 2004. Dynamics of a pasture soil microbial community after deposition of cattle urine amended with  $[^{13}C]Urea$ . *Appl. Environ. Microbiol.* 70, 6363–6369.
- Rodwell, J.S., 1992. *British Plant Communities Volume 3: Grasslands and Montane Communities*. Cambridge University Press.
- Smith, K.A., Ball, T., Conen, F., Dobbie, K.E., Massheder, J., Rey, A., 2003. Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. *Eur. J. Soil Sci.* 54, 779–791.
- Smith, K.A., Dobbie, K.E., Thorman, R., Watson, C.J., Chadwick, D.R., Yamulki, S., Ball, B.C., 2012. The effect of N fertilizer forms on nitrous oxide emissions from UK arable land and grassland. *Nutr. Cycl. Agroecosys.* 93, 127–149.
- Sugimoto, Y., Inoue, K., Nagamatu, K., Ueno, M., 1993. Nitrogen dynamics of urine in pasture: 1. Volatilization of ammonia from cattle urine patches. *J. Jpn. Soc. Grassl. Sci.* 39, 162–168.
- Terry, R.E., Tate III, R.L., Duxbury, J.M., 1981. The effect of flooding on nitrous oxide emissions from an organic soil. *Soil Sci.* 132, 228–232.
- Uchida, Y., Clough, T.J., Kelliher, F.M., Hunt, J.E., Sherlock, R.R., 2011. Effects of bovine urine, plants and temperature on  $N_2O$  and  $CO_2$  emissions from a sub-tropical soil. *Plant Soil* 345, 171–186.
- Urban, N.R., Eisenreich, S.J., Bayley, S.E., 1988. The relative importance of denitrification and nitrate assimilation in midcontinental bogs. *Limnol. Oceanogr.* 33 (6/2), 1611–1617.
- van Beek, C.L., Pleijter, M., Kuikman, P.J., 2011. Nitrous oxide emissions from fertilized and unfertilized grasslands on peat soil. *Nutr. Cycl. Agroecosys.* 89, 453–461.
- van Groenigen, J.W., Kuikman, P.J., de Groot, W.J.M., Velthof, G.L., 2005a. Nitrous oxide emission from urine-treated soil as influenced by urine composition and soil physical conditions. *Soil Biol. Biochem.* 37, 463–473.
- van Groenigen, J.W., Velthof, G.L., van der Bolt, F.J.E., Vos, A., Kuikman, P.J., 2005b. Seasonal variation in  $N_2O$  emissions from urine patches, effects of urine concentration, soil compaction and dung. *Plant Soil* 273, 15–27.
- Velthof, G.L., Koops, J.G., Duyzer, J.H., Oenema, O., 1996. Prediction of nitrous oxide fluxes from a managed grassland on peat soil using a simple empirical model. *Ned. J. Agric. Sci.* 44, 339–356.
- Velthof, G.L., Oenema, O., 1995. Nitrous oxide fluxes from grassland in the Netherlands. 2. Effects of soil type, nitrogen fertilizer application and grazing. *Eur. J. Soil Sci.* 46, 541–549.
- Worrall, F., Chapman, P., Holden, J., Evans, C., Artz, R., Smith, P., Grayson, R., 2011. A review of current evidence on carbon fluxes and greenhouse gas emissions from UK peatland. *JNCC Report*, No. 442.
- Wrage, N., van Groenigen, J.W., Oenema, O., Baggs, E.M., 2011. A novel dual-isotope labeling method for distinguishing between soil sources of  $N_2O$ . *Rapid Commun. Mass Spectrom.* 19, 3298–3306.
- Whitehead, D.C., 1986. Sources and transformation of organic nitrogen in intensively managed grassland soils. In: Van der Meer, H.G., Ryden, J.C., Ennik, G.C. (Eds.), *Nitrogen Fluxes in Intensive Grassland Systems*. Martinus Nijhoff, Dordrecht, pp. 47–55.
- Yamulki, S., Jarvis, S.C., Owen, P., 1999. Methane emission and uptake from soils as influenced by excreta deposition from grazing animals. *J. Environ. Qual.* 28, 676–682.